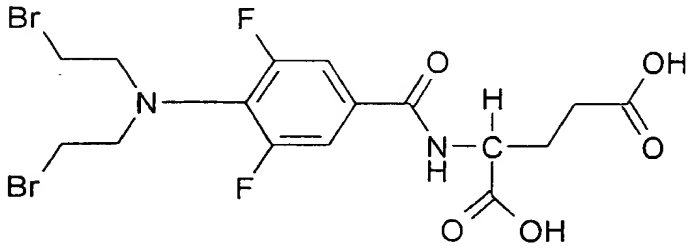
(ii) {3,5-difluoro-4-[bis(2-bromoethyl)amino]benzoyl}-L-glutamic acid

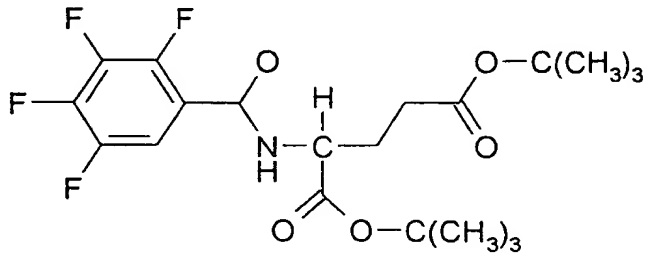


The product of Example 3(i) was dissolved in 10 ml TFA and treated as in Example 1(iv) to provide 0.32 g of crystalline product.

^{1}H NMR δ 1.95 + 2.05 (2m, 2H, CH_2CH), 2.35 (t, 2H, CH_2CO_2, J = 7.5Hz), 3.55 (t, 4H, CH_2N, J = 5.5Hz), 3.61 (t, 4H, CH_2Br, J = 5.5Hz), 4.4 (1H, m, CH), 7.60 (d, 2H, H2+6, J = 10Hz), 8.62 (d, 1H, NH, J = 7.5Hz); ^{19}F NMR δ -117.32 (d, 2F, F3+5, J = 10Hz); MS m/z 539 ($M+Na^+$, 24), 517 ($M+H^+$, 59), 423 (M-CH_2Br, 33), 370 (M-glu, 100). Anal. ($C_{16}H_{18}N_2O_5F_3Cl_2$.0.32toluene) C,H, N.

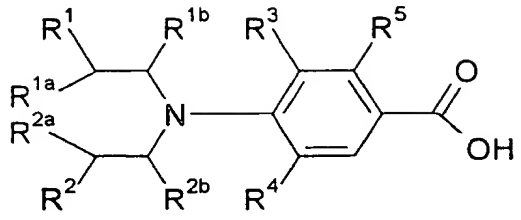
Example 4

(i) di-*tert*-butyl (2,3,4,5-tetrafluorobenzoyl)-L-glutamate



To an ice-cold solution of di-*tert*-butyl L-glutamate hydrochloride (6.6 g, 22.4 mmol) and Et_3N (6.9 ml, 50 mmol) in dry CH_2Cl_2 (70 ml) was added, over a period of 1.5 hr, 2,3,4,5-tetrafluorobenzoyl chloride (5.0 g, 23.5 mmol) in dry

21. A compound of Formula II:



wherein:

R^1 is -Cl, -Br, -I, $-OSO_2CH_3$, or $-OSO_2Ph$;

R^2 is -Cl, -Br, -I, $-OSO_2CH_3$, or $-OSO_2Ph$;

wherein Ph denotes a phenyl group which is optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from a C_{1-4} alkyl group, -F, -Cl, -Br, -I, -CN, or $-NO_2$;

R^{1a} is -H, a C_{1-4}alkyl group, or a C_{1-4}haloalkyl group ;

R^{2a} is -H, a C_{1-4}alkyl group, or a C_{1-4}haloalkyl group ;

R^{1b} is -H, a C_{1-4}alkyl group, or a C_{1-4}haloalkyl group ;

R^{2b} is -H, a C_{1-4}alkyl group, or a C_{1-4}haloalkyl group ;

R^3 is -F, -Cl, -Br, -I, $-OCHF_2$, $-C\equiv CH$, $-OCF_3$, $-CH_3$, $-CF_3$, $-SF_5$, $-SCF_3$, or $-CF_2CF_3$;

R^4 is -H, -F, -Cl, -Br, -I, $-OCHF_2$, $-C\equiv CH$, $-OCF_3$, $-CH_3$, $-CF_3$, $-SF_5$, $-SCF_3$, or $-CF_2CF_3$;

R^5 is -H or -F;

with the proviso that if R^4 is -H, then R^3 is not -F.

22. A compound according to claim 21, wherein:
R^1 and R^2 are independently -I, -Br, or -Cl.

23. A compound according to claim 21, wherein:
R^1 and R^2 are both -I.

24. A compound according to any one of claims 21 to 23, wherein:
R^{1a}, R^{1b}, R^{2a}, R^{2b} are each independently -H or $-CH_3$.

25. A compound according to any one of claims 21 to 23, wherein:
R^{1a}, R^{1b}, R^{2a}, R^{2b} are all -H.

26. A compound according to any one of claims 21 to 25, wherein:
R^3 and R^4 are $-CF_3$ and -H, respectively.

27. A compound according to any one of claims 21 to 25, wherein:
R^3 and R^4 are both -F.

28. A compound according to any one of claims 21 to 25, wherein:
R^3 and R^4 are $-CF_3$ and -H, respectively; and,
R^5 is -H.

29. A compound according to any one of claims 21 to 25, wherein:
R^3 and R^4 are both -F; and,
R^5 is -F.

30. A compound according to any one of claims 21 to 25, wherein:
R^3 and R^4 are both -F; and,
R^5 is -H.

31. 3,5-difluoro-4-[bis(2-iodoethyl)amino]benzoic acid.

32. 3,5-difluoro-4-[bis(2-chloroethyl)amino]benzoic acid.

33. 3,5-difluoro-4-[bis(2-bromoethyl)amino]benzoic acid.

34. 2,3,5-trifluoro-4-[bis(2-chloroethyl)amino]benzoic acid.

35. 2,3,5-trifluoro-4-[bis(2-bromoethyl)amino]benzoic acid.

36. 2,3,5-trifluoro-4-[bis(2-iodoethyl)amino]benzoic acid.

37. 3,5-difluoro-4-[bis(2-bromopropyl)amino]benzoic acid.

38. 3-trifluoromethyl-4-[bis(2-bromoethyl)amino]benzoic acid.

39. A two-component system comprising:
(i) a first component capable of delivering a carboxypeptidase enzyme to the interior or exterior of a target cell or a vector encoding said enzyme to the interior of said cell such that said vector expresses said enzyme in said cell, and
(ii) a prodrug of according to any one of claims 1 to 20 capable of being converted by said enzyme into a drug according to any one of claims 21 to 38.

40. A kit comprising:
(a) a compound according to any one of claims 1 to 20; and,
(b) one of:
(i) an immunoglobulin/enzyme fusion protein or conjugate in which the immunoglobulin is specific for a cellular antigen and the enzyme is a carboxypeptidase enzyme;
(ii) a ligand/enzyme conjugate or fusion protein, the ligand being specific for a cellular antigen and the enzyme is a carboxypeptidase enzyme;
(iii) a vector which encodes a carboxypeptidase enzyme which can be expressed in a cell.

41. A composition comprising a compound according to any one of claims 1 to 38, and a pharmaceutically acceptable carrier or diluent.

42. A compound according to any one of claims 1 to 38 for use in a method of treatment of the human or animal body.

43. A compound according to any one of claims 1 to 38 for use in a method of treatment of cancer of the human or animal body.

44. Use of a compound according to any one of claims 1 to 38 for the manufacture of a medicament for use in the treatment of cancer.

45. A method for the treatment of cancer comprising administering to a subject suffering from cancer a therapeutically-effective amount of a compound according to any one of claims 1 to 38.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 17 November 2000 (17.11.00)	
International application No. PCT/GB00/01194	**Applicant's or agent's file reference** WJW/BP5835335
International filing date (day/month/year) 29 March 2000 (29.03.00)	**Priority date** (day/month/year) 31 March 1999 (31.03.99)
Applicant SPRINGER, Caroline, Joy et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 October 2000 (13.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Zakaria EL KHODARY

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WJW/BP5835335		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01194	29/03/2000	31/03/1999
International Patent Classification (IPC) or national classification and IPC C07C237/36		
Applicant CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/10/2000	11.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Sen, A Telephone No. +49 89 2399 8328

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01194

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-45,47-58	as originally filed			
46	as received on	27/12/2000	with letter of	21/12/2000

Claims, No.:

1-20	as originally filed			
21-47	as received on	27/12/2000	with letter of	21/12/2000

Drawings, sheets:

1/1	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-47
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-47
Industrial applicability (IA)	Yes:	Claims	1-47
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION V:

1. The subject-matter of the application meets the requirements of Article 33(2) PCT since the prior art documents WO 94 25429 A (D1) and WO 97 03957 A (D2) do not describe the present nitrogen mustard compounds and prodrugs thereof of general Formulas I and II, respectively.

2. Document WO 94 / 25429 (D1) describes 2-fluoro- and 3-fluoro-substituted nitrogen mustard compounds wherein the substituent R_3 is F and the groups Y and L (present R^1 and R^2) are preferably both chloro groups, both mesyloxy or chloro and mesyloxy. Compounds of D1 have been disclaimed by means of a proviso. Because of the structural similarity between the compounds claimed and the compounds of the prior art D1, and because the compounds of D1 are also described as prodrugs for use "in a method of treatment of the human or animal body by therapy, particularly a method of treatment of cancer", an objection of lack of inventive step under Article 33(3) PCT is raised with regard to the present application.
The same objection is raised at the light of document WO 97 / 03957. This document describes site specific nitrogen mustards of general formula (III). These compounds "are known compounds and may be prepared through reactions well described in the organic chemistry". Accordingly, the inventive step under Art. 33(3) PCT for the present nitrogen mustard compounds claimed is not evident also because all the related prior art documents mention the compounds in connection with the use in the therapy and the treatment of cancer.

3. For the assessment of the present claim 47 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

SECTION VII:

1. Claims 13 to 20 and 22, 23, 33 to 40 contain all the features of claims 1 and 21,

respectively, and therefore have to be formulated as claims dependent on claims 1 and 21 (Rule 6.4 PCT).

2. To meet the requirements of Rule 5.1(a)(ii) PCT, the documents D1 and D2 should be identified in the description and the relevant background art disclosed therein should be discussed in its entirety.

SECTION VIII:

1. Inconsistency is noted between the subject-matter of claim 1 and claim 21. The definitions are not consistent so that a clear connection between the compounds of general formula II and the prodrug thereof (see Formula I) is not evident [see for example claim 41].

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
WJW/BP5835335		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 01194	29/03/2000	31/03/1999
Applicant		
CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1a

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 00/01194

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C237/36 A61K31/223 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 25429 A (CANCER RES CAMPAIGN TECH ;SPRINGER CAROLINE JOY (GB)) 10 November 1994 (1994-11-10) page 22	1,2,4,5, 11,12, 41-45
X	WO 97 03957 A (PHARMACIA & UPJOHN SPA ;COZZI PAOLO (IT); BERIA ITALO (IT); CAPOLO) 6 February 1997 (1997-02-06) page 14	21,22, 24,25

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2000	02/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bader, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/GB 00/01194

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9425429 A	10-11-1994	AT 172958 T	15-11-1998
		DE 69414379 D	10-12-1998
		DE 69414379 T	12-05-1999
		EP 0696270 A	14-02-1996
		ES 2125450 T	01-03-1999
		JP 8509490 T	08-10-1996
		US 5811454 A	22-09-1998
WO 9703957 A	06-02-1997	AU 6357996 A	18-02-1997
		BR 9606528 A	23-12-1997
		CA 2199635 A	06-02-1997
		CN 1159183 A	10-09-1997
		EP 0787126 A	06-08-1997
		HU 9702393 A	28-04-1998
		JP 10506410 T	23-06-1998
		NO 971142 A	12-03-1997
		PL 319352 A	04-08-1997